Augmented inducible nitric oxide synthase expression and increased NO production reduce sepsis-induced lung injury and mortality in myeloperoxidase-null mice

Viktor Brovkovych,1,2* Xiao-Pei Gao,1,2* Evan Ong,1,2 Svitolana Brovkovych,1,2 Marie-Luise Brennan,3 Xiao Su,1,2 Stanley L. Hazen,3 Asrar B. Malik,1,2 and Randal A. Skidgel1,2

1Department of Pharmacology and 2Center for Lung and Vascular Biology, University of Illinois College of Medicine, Chicago, Illinois; and 3Departments of Cell Biology and Cardiovascular Medicine, Cleveland Clinic Foundation, Cleveland, Ohio

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Address for reprint requests and other correspondence: R. A. Skidgel, Dept. of Pharmacology (MC 868), Univ. of Illinois College of Medicine, 835 S. Wolcott, Chicago, IL 60612 (e-mail: rskidgel@uic.edu).


Augmented inducible nitric oxide synthase expression and increased NO production reduce sepsis-induced lung injury and mortality in myeloperoxidase-null mice (10, 34). Low levels of NO enhance MPO activity, whereas high levels can inhibit MPO catalysis due to formation of nitrosyl complexes (1, 2). MPO activity in lungs of iNOS-deficient mice is reduced in acute inflammation compared with control mice (9). Conversely, MPO affects NO signaling in multiple ways. For example, MPO is secreted by activated PMNs (32) and undergoes transcytosis across the vessel wall where it oxidizes NO and reduces its bioavailability in tissue (11, 38). MPO consumption of NO increases iNOS activity by reducing NO-mediated feedback inhibition (13). MPO also utilizes the NO breakdown product, NO2−, as a physiological substrate, producing NO-derived ROS that promote tyrosine nitration and lipid peroxidation (30, 43). Mycoplasma infection reduces alveolar fluid clearance by inhibiting epithelial Na+ channels via ROS and nitrogen species in wild-type (WT) mice but not in MPO−/− mice (19). LPS challenge also impairs NO-dependent vascular responses in control mice but not in MPO−/− mice (11).

The response to infection is perturbed in patients with deficiencies of the enzyme-based host defense system. Patients with chronic granulomatous disease, in which NADPH oxidase deficiencies of the enzyme-based host defense system. Patients with chronic granulomatous disease, in which NADPH oxidase is defective, are extremely susceptible to bacterial infection (33). In contrast, MPO-deficient patients generally do not have increased frequency of infections (26) despite impaired HOCl production (15). These findings suggest that compensatory mechanisms can confer resistance in the absence of MPO. In the present study, we investigated the relationship between MPO and iNOS in MPO−/− mice using a model of Escherichia coli-induced septicemia. We addressed the possibility that iNOS plays a crucial compensatory role that maintains host defense in the absence of MPO. We observed markedly increased basal iNOS expression and NO production that underlie the ability of MPO−/− mice to resist bacterial infection. Thus, strikingly, lung inflammatory injury was greatly reduced in the absence of the MPO-hydrogen peroxide-halide host defense pathway in MPO−/− mice.

Materials and Methods

Animals. Studies were made using MPO−/− mice (5), and wild-type C57BL/6 mice (WT; The Jackson Laboratory, Bar Harbor, ME) were used as controls. Mice (22–26 g, 9–12 wk old) were housed in specific pathogen-free conditions with free access to food and water in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
the University of Illinois Animal Care Facility. All experimental procedures complied with institutional and National Institutes of Health guidelines for animal use, and approvals were obtained from Animal Care Committee of the University of Illinois at Chicago.

E. coli infection and survival studies in mice. Live E. coli were obtained from American Type Culture Collection, Manassas, VA (ATCC 25992). Mice were challenged intraperitoneally with 1 x 10^9 live E. coli for survival studies and with 0.5 to 2 x 10^9 live E. coli for other experiments as indicated. Control mice were injected intraperitoneally with an equal volume of PBS.

Pulmonary microvascular permeability. Pulmonary capillary filtration coefficient (Kf) was measured to determine pulmonary microvascular permeability to liquid as previously described (28). After the standard 20-min equilibration perfusion, the outflow pressure was rapidly elevated by 10 cmH2O for 2 min, and the lung wet weight was monitored. Lung dry weight was determined, and Kf (ml min^-1 cmH2O^-1 dry g^-1) was calculated from the slope of the recorded weight change normalized to the pressure change and lung dry weight. Pulmonary edema formation was measured by continuously monitoring the lung wet weight change for 90 min (36). Because perfusate albumin concentration was constant, and pulmonary arterial pressure did not change, the rate and magnitude of the increase in lung wet weight provided another index of pulmonary microvesSEL permeability.

The effect of iNOS inhibitor L-NAME-(1-iminoethyl)lysine (L-NIL) on lung edema in vivo in WT and MPO^-/- mice treated with E. coli was determined by measuring excess lung water. Lungs were excised, weighed, and then homogenized after the addition of 1 ml of double-distilled H2O. The homogenate was centrifuged at 12,000 rpm for 10 min to obtain the supernatant, and the hemoglobin content was determined. Hemoglobin content and hematocrit were determined in a blood sample from the right ventricle. Fractions of homogenate, supernatant, and blood were weighed and then placed in a drying oven at 60°C for 24 h after which the dry weights were determined. The lung wet-to-dry ratio and final excess lung water were calculated as previously described (37).

Lung PMN sequestration. Lung PMN sequestration was assessed by determining MPO activity and also morphometrically quantifying PMN infiltration as previously described (28). Mouse lungs were inflated with 10% formalin and embedding in paraffin. Hematoxylin and eosin-stained tissue sections were visualized using a high magnification (x100) objective with an oil-immersion numerical aperture. Middle region (~30 mm^2) of the left lung upper lobe was outlined at low magnification (~x125). At least 5% of the outlined region was measured with a systematic random design of counting frames. The total number of PMNs in the outlined region of lung was determined using the formula n = ΣQ^- × section sampling fraction (SSF)/area sampling fraction (ASF), where ΣQ^- is the total number of PMN counted by optical evaluation using a random design procedure. The ASF is the counting frame (6,400 μm^2), and SSF is the fraction of section sampled in the region of the lung.

PMN isolation. PMNs from WT or MPO^-/- mice were purified from peripheral venous blood after collection into EDTA anticoagulant and 1:1 dilution into Ca^2+/?Mg^2+?-free HBSS-BSA, followed by a discontinuous Percoll gradient as previously described (14, 27). This procedure yielded PMN purity of 90–95% and >95% viability assessed by trypan blue exclusion.

Measurement of nitrite concentration. PMNs from peripheral blood of WT or MPO^-/- mice were incubated in arginine (Arg)-free DMEM for 1 h. Fresh DMEM was added containing 1 mM Arg, and cells were then incubated for 3 h at 37°C. Accumulated nitrite + nitrate in the medium of PMNs or in plasma isolated from WT or MPO^-/- mice was measured after reduction of nitrate to nitrite using a Cd-Cu Reducer (Nitralyzer II kit, World Precision Instruments) following the manufacturer’s instructions. Nitrite was measured in 50-μl aliquots using the Griess reagent (nitrite detection kit; Promega).

Measurement of lung tissue iNOS activity. Lung slices (1-mm thick) were washed and placed in HBSS with or without 1 mM Arg at 37°C. NO production was measured in real-time using a three-electrode system (6, 36); a porphyrinic microsensor supercoated with Nafion polymer, a platinum counter electrode, and a silver-silver chloride reference electrode. A micromanipulator was used to place the microsensor on the surface of the lung slice, and the baseline was recorded. To determine constitutive NOS (cNOS) activity, lung slices in HBSS containing 1 mM Arg were stimulated with 10 μM calcium ionophore A-23187. To measure the lung iNOS activity, slices were incubated in HBSS without l-arginine (l-Arg) for 60 min, and NO generation was initiated by application of 1 mM l-Arg.

Lung microvascular permeability and edema formation. Water content was continuously monitored in perfused lungs from control animals or mice challenged for 6 h with E. coli 10^9 cells). All mice were alive at 6 h after this dosage. The weight of lungs from control WT or MPO^-/- mice remained stable over the 90-min perfusion, whereas lungs from WT mice treated with E. coli had significantly increased water accumulation after 60 min of monitoring, which continued to
ENHANCED iNOS ACTIVATION IN MPO DEFICIENCY

Fig. 1. A: Kaplan-Meier survival plots for wild-type (WT) and myeloperoxidase MPO−/− mice. Mice were challenged with 10⁸ Escherichia coli per mouse via intraperitoneal injection. Survival curves were generated from 2 independent experiments with a total of 20 mice per group. The difference in survival between the WT and MPO−/− mice was significant (P = 0.02 by the log-rank test). B: Changes in lung wet weight (grams). WT and MPO−/− mice were unchallenged or challenged with 10⁸ E. coli intraperitoneally for 6 h. Lungs were removed and perfused, and lung wet weights were continuously monitored. Points indicate means ± SE (n = 5). *P < 0.05 compared with untreated WT mice. C: Lung microvascular permeability (measured as capillary filtration coefficient, Kf,c), a measure of vascular permeability. WT and MPO−/− mice demonstrated similar lung microvascular permeability values under basal condition (Fig. 1C). However, at 6 h after E. coli challenge, Kf,c increased significantly in both groups, but the response was threefold greater in lungs from WT than MPO−/− mice (Fig. 1C).

PMN sequestration in lungs. After E. coli challenge, lung tissue MPO activity (a measure of PMN sequestration) in WT mice increased sixfold at 6 h; predictably, MPO activity was not detected in lungs from MPO−/− mice (data not shown). Thus we determined PMN sequestration in lungs of MPO−/− mice by morphometric analysis (Fig. 1D). PMN uptake in

Fig. 2. A: plasma nitrite + nitrate accumulation in WT and MPO−/− mice after E. coli challenge. Plasma nitrite + nitrate levels were measured before or 6 h after E. coli challenge (10⁸ E. coli). Bars indicate means ± SE (n = 5). *P < 0.05 compared with WT before E. coli challenge. B: NO production from isolated peripheral blood PMN before and after E. coli challenge. MPO−/− mice demonstrated almost no wet weight gain (Fig. 1B). This difference was particularly striking at 90 min. We determined pulmonary capillary filtration coefficient (Kf,c), a measure of vascular permeability. WT and MPO−/− mice demonstrated similar lung microvascular permeability values under basal condition (Fig. 1C). However, at 6 h after E. coli challenge, Kf,c increased significantly in both groups, but the response was threefold greater in lungs from WT than MPO−/− mice (Fig. 1C).
lungs of MPO−/− mice increased ninefold at 6 h after E. coli challenge, which was similar to the response in lungs of WT mice (Fig. 1D).

**NO production.** Blood plasma nitrite + nitrate concentration, the stable NO breakdown products, in MPO−/− mice before E. coli challenge was twofold greater than WT mice (Fig. 2A). At 6 h after E. coli challenge, plasma nitrite + nitrate concentration in WT mice increased twofold. E. coli challenge of MPO−/− mice slightly increased the nitrite + nitrate concentration, and it rose to the same final level as in WT mice (Fig. 2A). Because plasma nitrite + nitrate concentration only provides a general index of overall NO production that can be derived from several sources, we directly evaluated NO production and iNOS protein expression in two important targets, peripheral blood PMNs and lungs of WT and MPO−/− mice before and after E. coli challenge. To measure iNOS, which, once expressed, has calcium-independent constitutive activity that requires extracellular L-Arg (8), PMNs were incubated in medium containing 1 mM L-Arg and then treated with calcium ionophore A-23187. This resulted in rapid increase of NO production that returned to baseline in 20 s (Fig. 3A). To measure the Ca2+-independent iNOS activity, lung slices were preincubated in Arg-free medium to stop basal iNOS activity. L-Arg (1 mM) was then added to initiate iNOS-mediated NO production followed by incubation for an additional 3 h. Basal NO2− + NO3− produced by control PMNs from MPO−/− mice was sixfold greater than WT PMNs. E. coli challenge resulted in significant increase in NO production in both cases, but the increase was much greater in WT mice (Fig. 2B). Western blot analysis of iNOS protein showed PMNs from MPO−/− mice had much higher iNOS expression than WT mice and that E. coli challenge significantly increased in iNOS expression in both WT and MPO−/− PMNs with a larger increase seen in WT mice (Fig. 2C).

**NO production in lungs of WT and MPO−/− mice.** NO released from lungs could be an alternative source of increased NO2− in the blood of MPO−/− mice. To measure Ca2+-regulated constitutive isoforms e/nNOS (cNOS), lung slices were incubated in medium containing 1 mM L-Arg and then treated with calcium ionophore A-23187. This resulted in rapid increase in NO production that returned to baseline in 20 s (Fig. 3A). To measure the Ca2+-independent iNOS activity, lung slices were preincubated in Arg-free medium to stop iNOS activity, and then 1 mM L-Arg was added to initiate NO production. This resulted in a marked and prolonged increase in NO concentration still increasing at 20 min (Fig. 3B).
followed by a plateau at 45–60 min and then slowly declining to baseline by 90 min (data not shown). Although the peak concentration of NO achieved by eNOS activity was similar to that derived from iNOS-dependent activity at 20 min, the total output of iNOS-derived NO was much greater because of the prolonged time course compared with the short, 20-s burst provided by eNOS (Fig. 3). eNOS activity in lungs of WT or *MPO*−/− mice was similar and did not change after *E. coli* challenge (Fig. 3A). In contrast, basal iNOS activity was twofold greater in lungs from *MPO*−/− mice compared with WT, and *E. coli* challenge in both cases resulted in significant increases in iNOS-dependent NO production (Fig. 3, B and C). Western blot analysis (Fig. 3D) showed that the basal iNOS protein level in lung tissue from *MPO*−/− mice was greater than WT and that *E. coli* challenge induced similar final levels of iNOS protein in lungs of WT and *MPO*−/− mice. In contrast, eNOS protein levels were similar in WT and *MPO*−/− lungs and did not increase after *E. coli* (data not shown), similar to our previous findings (36).

**Nitrotyrosine generation in lungs.** Nitrotyrosine is generated by peroxynitrite (ONOO−) and ·NO2, an oxidant produced by MPO conversion of NO2− (12, 17). We evaluated nitrotyrosine production in lungs from WT and *MPO*−/− mice. The ratio of NO2−-Tyr/Tyr increased from 35 ± 7 to 66 ± 8 1 h after *E. coli* challenge in WT lungs (Fig. 3E). In contrast, basal NO2−-Tyr/Tyr ratio in *MPO*−/− mice did not differ from WT and did not increase 1 h after *E. coli* (Fig. 3E) indicating that MPO plays an important role in nitrotyrosine formation.

**Effects of iNOS inhibition.** Treatment of WT mice with different doses of the iNOS-specific inhibitor L-NIL 20 min before *E. coli* challenge induced concentration-dependent decreases in sepsis-induced accumulation of plasma nitrate + nitrite (Fig. 4A) with a maximum effect seen at 5 mg/kg. Pretreatment with 5 mg/kg L-NIL did not change basal nitrite + nitrate levels in WT mice but reduced the elevated plasma nitrite + nitrate concentration in *MPO*−/− mice to that of WT mice (Fig. 4B), indicating that high basal nitrite + nitrate concentration in *MPO*−/− mice is derived from iNOS activity. L-NIL pretreatment also blocked nitrite + nitrate accumulation in response to *E. coli* in both WT and *MPO*−/− mice (Fig. 4B). Treatment with L-NIL also blocked iNOS activity in lungs of control *MPO*−/− mice and in WT and *MPO*−/− mice after *E. coli* challenge (Fig. 4C).

Next, we compared the bacterial clearance capacity of WT and *MPO*−/− mice. The bacterial burden in lungs at 1 or 6 h after *E. coli* challenge was significantly less in *MPO*−/− mice compared with WT (Fig. 4D). Pretreatment with iNOS-specific inhibitor L-NIL significantly reduced bacterial colony counts 6 h after *E. coli* in WT mice, whereas in *MPO*−/− mice, the same pretreatment increased bacterial colony counts at 1 and

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**Fig. 4.** A: nitrite + nitrate production in blood plasma of WT mice after *E. coli* challenge. Different doses of L-N^6-(1-iminoethyl)lysine (L-NIL) were administrated to WT mice 20 min before *E. coli* challenge, and blood plasma levels were measured at 6 h after *E. coli*. Bars indicate means ± SE (n = 3). **P < 0.05 vs. control; *P < 0.05 vs. 0 mg/kg L-NIL. B: effects of iNOS inhibition on nitrite + nitrate levels in plasma of WT and *MPO*−/− mice after *E. coli* challenge. WT and *MPO*−/− mice were pretreated without or with 5 mg/kg L-NIL for 20 min, and nitrite + nitrate levels were measured before or 6 h after *E. coli* challenge. L-NIL blocked the increase in plasma nitrite + nitrate in WT mice after *E. coli* and reduced basal and post-*E. coli* nitrite + nitrate levels in *MPO*−/− mice. Bars indicate means ± SE (n = 4–6). **P < 0.05 vs. without L-NIL pretreatment; **P < 0.05 vs. WT, no *E. coli*. C: iNOS activity in lungs from mice treated as in B was measured in real-time using a porphyrimic microsensor as described in MATERIAL AND METHODS. Data are presented as relative area under NO curve for 20 min. Bars indicate means ± SE (n = 4–6). **P < 0.05 vs. without L-NIL pretreatment; **P < 0.05 vs. WT, no *E. coli*. D: lung bacterial clearance at 1 and 6 h following intraperitoneal administration of *E. coli* [2 × 10^8 colony-forming units (CFU)] in WT and *MPO*−/− mice were measured as described in MATERIALS AND METHODS. Bars indicate means ± SE (n = 5). **P < 0.05 vs. corresponding point without L-NIL treatment; #P < 0.05 vs. corresponding point in WT mice.
6 h compared with mice without iNOS inhibitor (Fig. 4D). These data suggest a role for iNOS-derived NO in bacterial clearance in MPO−/− mice.

To determine the role of iNOS-derived NO on lung injury, we measured the effect of E. coli challenge on excess lung water in animals treated with the iNOS inhibitor L-NIL. E. coli treatment resulted in increased lung edema in WT mice but did not cause a significant increase in MPO−/− mice (Fig. 5A). Interestingly, inhibition of iNOS led to a decrease in lung edema in WT mice after E. coli treatment, whereas in MPO-null mice, L-NIL resulted in increased edema (Fig. 5A), consistent with the effects of iNOS inhibition on bacterial clearance (Fig. 4D).

Because MPO deletion conferred a significant survival advantage to lethal E. coli challenge (Fig. 1A), we investigated the effect of iNOS inhibition on the response. Pretreatment of WT mice with L-NIL resulted in an increase in survival, whereas pretreatment of MPO−/− mice with L-NIL resulted in decreased survival (Fig. 5B). Taken together, these data suggest a role for iNOS-derived NO in MPO−/− mice that results in increased bacterial clearance, reduced lung injury, and increased survival in this model of E. coli-induced sepsis.

DISCUSSION

NADPH oxidase, MPO, and iNOS are important enzymes regulating antimicrobial host defense (see Refs. 22, 31, 41). MPO produces HOCl, a potent bactericidal agent that is also a proinflammatory oxidant capable of inducing tissue injury and inflammation (15, 22, 42). MPO also catalyzes nitrotyrosine formation and lipid peroxidation by converting NO2− to the potent oxidant ·NO2 (22, 30, 43). Despite the presumed importance of MPO in host defense, patients with MPO deficiency exhibit almost normal resistance to infection (15, 26). Although this led to claims that MPO does not play a crucial role in host defense, a more likely explanation is that compensatory mechanisms substitute for its loss (16, 22). However, the nature and relative importance of these compensatory effects remain controversial (16, 22).

In the present study, we used MPO-null mice to investigate mechanisms responsible for protection of the host in the absence of a functional peroxide-halide pathway. We observed, surprisingly, that MPO−/− mice had enhanced bacterial clearance, reduced lung injury and edema formation, as well as greater survival following E. coli challenge compared with WT mice. The results show that increased iNOS expression and NO production in MPO−/− mice were crucial in mitigating the effects of MPO deficiency. Several lines of evidence support this contention. First, basal expression of iNOS and NO production were significantly enhanced in PMNs and lungs of MPO−/− mice as reflected in the higher iNOS protein levels, iNOS activity in lung tissue and isolated PMNs, and plasma NO2 levels. Second, bacterial colony counts in lungs of MPO−/− mice were significantly lower than in WT mice at both 1 and 6 h after E. coli administration. Third, inhibition of iNOS significantly increased bacterial colony counts in lungs of MPO−/− mice after E. coli administration. Fourth, inhibition of iNOS significantly reduced the survival of MPO−/− mice in response to E. coli challenge.

High basal expression of iNOS in MPO-null mice could help to explain differences in survival seen in MPO−/− and WT mice after E. coli challenge. Lungs and PMNs of MPO−/− mice constitutively produced higher levels of NO than WT mice. The preexisting higher level of NO, which has antimicrobial activity (29, 40), may act to kill or slow the replication of the E. coli from the time of administration compared with WT mice in which iNOS upregulation was not maximal until 6 h. NO itself is generally not toxic to host tissue but may become damaging in combination with ROS (30), which would...
become prominent at later times during sepsis. Thus the high NO existing before E. coli challenge in MPO\(^{-/-}\) mice in this case would be protective at the early stage of sepsis, whereas in WT mice, high NO generated later in the response could combine with ROS that are also present to cause host tissue damage (30). This possibility is consistent with our present finding that inhibition of iNOS results in less lung injury and increased survival in WT mice as well as our previous study (36) showing that NOS inhibition reduced macrophage inflammatory protein-2 (MIP-2) expression and lung edema associated with E. coli-induced sepsis in WT mice. Reactive oxygen and nitrogen species were also shown to impair the ability of the lung to clear alveolar fluid by inhibiting epithelial Na\(^{+}\) channels in mycoplasma-infected mice (19). Interestingly, MPO\(^{-/-}\) mice maintained their ability to clear alveolar fluid after mycoplasma infection, likely due to lack of generation of reactive oxidants (19), consistent with our findings of reduced reactive oxygen and nitrogen species were also shown to impair the ability of tissue injury and edema in response to E. coli infection in MPO-deficient mice.

MPO-derived \(\cdot\)NO\(_2\) causes tyrosine nitration as well as lipid peroxidation, resulting in alteration of protein function, cell membrane injury, and generation of proinflammatory mediators (30, 43). Because NO terminates lipid peroxidation (20), and MPO scavenges and inactivates NO (1), MPO\(^{-/-}\) mice are expected to have reduced lipid peroxidation. In our studies, we observed that E. coli challenge resulted in a marked increase in nitrotyrosine production in lungs of WT mice, but no increase was seen in MPO\(^{-/-}\) mice. In addition to MPO-derived \(\cdot\)NO\(_2\), nitrotyrosine is generated from reaction of tyrosine residues with ONOO\(^-\) derived from NO and superoxide (30). It was shown that MPO\(^{-/-}\) mice injected intraperitoneally with thioglycolate or zymosan produced significantly less nitrotyrosine compared with WT mice (43). Thus, in some inflammatory models, MPO appears to play a dominant role in nitrotyrosine formation. In the E. coli sepsis model in the present study, however, ONOO\(^-\) did not appear to contribute to nitration of tyrosine in MPO\(^{-/-}\) lungs at 1 h after E. coli challenge despite high basal expression of iNOS and NO production. This finding helps to explain the reduction in lung inflammatory injury and mortality in MPO\(^{-/-}\) mice compared with WT mice. In addition, the lack of HOCl production during sepsis in MPO\(^{-/-}\) mice could reduce tissue injury compared with WT (22). These data are consistent with a previous study showing no difference in lung nitrotyrosine formation between WT and iNOS\(^{-/-}\) mice after mycoplasma infection and a reduction in nitrotyrosine to uninfected levels in mice depleted of PMNs by cyclophosphamide, indicating the importance of PMN-derived MPO in this process (18). MPO also oxidizes glutathione (39), an important cellular antioxidant; thus augmentation of this antioxidant defense in MPO\(^{-/-}\) mice could be another factor contributing to their protection.

We considered the possibility that alterations in eNOS could have contributed to the higher NO production and protection seen in MPO\(^{-/-}\) mice. Studies have reported varying effects of inflammation on eNOS expression (7). For example, eNOS promoter activity and mRNA expression were reduced by TNF-\(\alpha\) in cultured bovine endothelial cells (3) and human endothelial cells (25), whereas IFN/\(\gamma\)/LPS treatment of bovine endothelial cells resulted in increased expression of eNOS mRNA (21). However, in our previous study (36) and the present study, we observed that eNOS immunoreactivity in mouse lungs remained constant for up to 6 h after in vivo E. coli challenge, indicating that eNOS expression is not altered in response to gram-negative septicemia. In addition, the iNOS-specific inhibitor, \(1\) -NIL, blocked the excess NO production in MPO\(^{-/-}\) mice. Thus the present results cannot be explained by alterations in the activity of eNOS.

A key unresolved question is the mechanism of upregulation of iNOS expression in the MPO\(^{-/-}\) mice. Studies showed that MPO inhibited iNOS expression in IFN-\(\gamma\)/LPS-treated monocytic macrophages, and it was proposed that MPO scavenges the low levels of NO that are necessary for the induction of iNOS (24). It is thus possible that iNOS is upregulated in MPO\(^{-/-}\) mice secondary to absence of such an inhibitory effect of MPO on iNOS expression. However, this question has not been addressed in the MPO-null mouse model. In summary, our data show that augmentation of iNOS-dependent NO production in the absence of MPO is a crucial compensatory mechanism regulating host defense and thus prevents lung inflammation and injury induced by E. coli septicemia in MPO-null mice.

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